REMARKS

Claims 1, 3, 6, 7 and 9-12 were pending in the application at the time the Office Action was mailed. Claims 7, 9 and 10 were withdrawn from prosecution. Claims 1, 3, 6, 11 and 12 were rejected. By this amendment, claims 1 and 7 have been amended. No claims have been added or canceled. Therefore, claims 1, 3, 6, 7, and 9-12 are pending in the application. No new matter was added by virtue of these amendments and entry is respectfully requested. A Request for Continued Examination (RCE) is filed herewith.

Telephonic Interview

Applicants thank the examiner for the helpful and informative telephonic interview on March 6, 2009. During the interview, the examiner provided several suggestions for attempting to overcome the outstanding 103 rejections based on Zemlan.

Claim Rejections Under 35 U.S.C. 112, First Paragraph

Claims 1, 3, 6, 11, and 12 were rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. According to the Office Action, "the metes and bounds of the claims are indefinite due to the lack of recitation of volume to be analyzed in the instant methods for detecting quantities as low as 50pg." The Office Action further states that the grounds of this rejection could be obviated by amending the claims to recite "wherein NF-H can be detected in quantities as low as 50pg in 50μl."

Claim 1 (from which claims 3, 6, 11 and 12 depend) has been amended herein to recite "wherein NF-H can be detected in quantities as low as 50 pg in 50 μl" per the examiner's recommendation solely to expedite prosecution. Applicants neither agree with nor acquiesce in the rejection.

Accordingly, withdrawal of this rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. § 103

Claims 1, 3 and 6 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Hu et al. in view of Zemlan. Claims 1, 3, 6 and 11 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Hu et al. in view of Zemlan and further in view of Grainger et al. (US Patent No. 5,595,722). Claims 1, 3, 6 and 12 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Hu et al in view of Zemlan and further in view of Posmantur et al.

Claim 1 (from which claims 3, 6, 11 and 12 depend) as amended herein recites a "method of detecting neuronal injury in a subject, the method comprising the steps of: (a) providing a blood, serum, or plasma sample from the subject; (b) contacting the blood, serum, or plasma sample with an antibody that specifically binds to NF-H in the sample; (c) detecting the presence or amount of NF-H in the sample, wherein NF-H can be detected in quantities as low as 50 pg in 50 µl; and (d) correlating the presence or amount of NF-H in the sample with the neuronal injury." Applicants respectfully assert that the cited combinations of references fail to render the claimed invention obvious for several reasons. First, the combinations of references fail to teach all claim limitations, e.g., "NF-H can be detected in quantities as low as 50 pg in 50 µl." Second, the Zemlan reference which is relied upon for all rejections is a nonenabling reference. Zemlan discloses an ELISA for tau protein, which is an entirely different protein unrelated to NF-H. Applicants submit that NF-H leakage from CSF to blood could be distinguished from tau leakage. For several reasons, NF-H is much less likely to leave an injury site than tau. NF-H is part of the most insoluble complement of the axon, the neurofilament, a stable protein complex formed from strong interactions between multiple alpha-helical coiled coils. This alpha-helical coiled coil interaction is so strong that NF-H can only be separated from the other neurofilament subunits by chaotropic agents such as SDS or Urea. This means that in a cellular context NF-H is present in the form of at least a heterotetramer containing other neurofilament subunits. In contrast, tau associates with microtubules, much more labile structures than neurofilaments. Microtubules disassemble rapidly in the cold, if the GTP level declines and in a host of other situations including damage and disease states, most of which have little if any affect on neurofilaments. The tau protein, in contrast to the NF-H molecule, contains no alpha-helical region and has no tendency to dimerize under normal conditions. In addition, human NF-H

isoforms are 1026-1032 amino acids in length so even as monomers are much larger than the various isoforms of tau which are only 352-441 amino acids long. Finally, and in contrast to tau, NF-H is relatively resistant to cellular proteases, whereas Zemlan has shown that the form of tau released into CSF flowing brain injury is even smaller than 352-441 amino acids, a so far uncharacterized proteolytic fragment of tau. There is therefore a strong *a priori* case to expect tau and tau fragments to be generated and released much more readily from damaged or degenerating axons than NF-H.

Clearly, Zemlan is not an enabling reference with regard to a method of detecting neuronal injury in a subject including detecting the presence or amount of NF-H in a blood, serum, or plasma sample having a sensitivity as low as 50 pg in 50 µl (as recited in claim 1 as amended herein). Combining this nonenabling reference with Hu et al., Grainger et al., and Posmantur et al. would not result in the claimed invention. One of skill in the art combining the teachings of Hu et al., Grainger et al., Posmantur et al. and Zemlan would not have a reasonable expectation of success of yielding an assay for detecting NF-H in blood, serum or plasma samples having a sensitivity as low as 50 pg in 50 µl due to the unpredictability of detecting NF-H in blood, serum or plasma samples at such low concentrations and because Zemlan is a nonenabling reference. Zemlan includes *no* data regarding neurofilament proteins – from any bodily fluid. Zemlan describes experiments involving only tau proteins. As mentioned above, tau and NF-H are *entirely different* proteins. Applicant respectfully submits that the "working example" the examiner refers to on page 4 of the Office Action is not germane to the claimed invention, as this "working example" involved experimentation only with tau protein – not NF-H protein, which is an *entirely different and unrelated* protein with quite different size, function, multimerization, solubility, binding properties and evolutionary origin.

Any combination of Hu et al., Grainger et al., Posmantur et al., and Zemlan does not render the claimed invention obvious for the reasons set forth above. The cited combinations of Hu et al., Zemlan, Grainger et al. and Postmantur et al. fails to teach or suggest all claim limitations, and fails to provide any guidance to one of skill in the art how to detect neuronal injury in a subject by detecting NF-H in a blood, serum, or plasma sample in quantities as low as

50 pg in 50 µl. Applicants' discovery and development of the claimed invention was not straightforward, and was not obvious in view of the prior art.

Withdrawal of these rejections is therefore respectfully requested.

CONCLUSION

The currently pending claims before the examiner are supported throughout the specification and are patentable over the prior art. No new matter has been added. This application is now in full condition for allowance, and such action is respectfully requested.

A Request for a Retroactive Extension of Time and an RCE are filed herewith. A credit card payment is made herewith for the required fees. However, the Commissioner for Patents and Trademarks is hereby authorized to charge any underpayment of fees or credit any overpayment of fees to Deposit Account No. 14-1437.

The examiner is cordially invited to call the undersigned if clarification is needed on any matter within this amendment, or if the examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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